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TITLE: Enhancing the immunogenicity of a dengue-2 DNA vaccine with adjuvants and anti-FCγRI antibodies

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13. ABSTRACT *(Maximum 200 Words)*

The overall objective of the project is to use adjuvants to enhance the immunogenicity of a dengue DNA vaccine. The adjuvants to be evaluated in this project include aluminum phosphate, tetanus toxoid and anti-FcγR monoclonal antibodies chemically linked to the DNA vaccine. The protocol for linking the monoclonal antibodies to the dengue DNA vaccine was finalized. Anti-FcγRI antibodies were obtained from Accurate Chemical, Wesbury, NY. Anti- FcγRII and -FcγRIII monoclonal antibodies were also obtained and evaluated. Indirect immunofluorescence assays (IFA) were performed to confirm recognition of Fcγ receptors on K562 cells (human monocyte cell line) and P388D1 cells (mouse monocyte cell line) prior to linking to the dengue DNA vaccine. Experimental results indicated poor recognition of the Fcγ receptors by these monoclonal antibodies. Anti-FcγRI and anti-FcγRII monoclonal antibodies were obtained from Pharmingen Inc., San Diego, CA and evaluated. IFA confirmed recognition of the anti- FcγRII receptors but not FcγRI receptors on K562 cells. The P388D1 mouse cells showed no reactivity with either antibody. IFA antibody binding results are currently being confirmed by flow cytometry analysis. Upon final approval of the animal use protocol, mouse studies will begin to evaluate the adjuvant effects of aluminum phosphate and tetanus toxoid.

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dengue, DNA vaccine, monoclonal antibodies, non-human primates

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INTRODUCTION

Department of Defense (DoD) researchers have used the DNA vaccine approach to develop experimental vaccines against DEN-1 and DEN-2. These vaccines contain the envelope (E) and premembrane (prM) genes of the viruses. The E gene was chosen for inclusion in the DNA vaccines because monoclonal antibody mapping and recombinant protein immunization studies with flaviviruses have shown that the E gene contains the major epitopes responsible for eliciting neutralizing antibodies. The prM gene was also included in the vaccine because the expressed protein is thought to prevent low pH-induced irreversible conformational changes in the E protein as it is processed through the acidic compartments of the Golgi complex prior to secretion. The DEN-2 and DEN-1 DNA vaccines have been tested in mice and shown to elicit neutralizing antibody responses.

Two primate DNA immunization/challenge studies were completed. One utilized Rhesus monkeys, the other *Aotus* monkeys. Both studies were very similar in design and outcome. One-third of the animals were injected with control DNA (plasmid that does not express prM/E) and two-thirds of the animals were immunized with the DNA vaccine that expresses the prM/E genes of DEN-1 virus. All of the animals that received the DEN-1 DNA vaccine responded by producing neutralizing antibodies. The antibody levels peaked one month after the last DNA boost and then steadily declined during the following five months at which time the animals were challenged with DEN-1 virus. Upon challenge with live dengue virus, the DEN-1 DNA vaccine-immunized monkeys showed a significant reduction in the mean days of viremia compared to naïve controls, but the protection was not complete. Although successful, these studies do indicate the need to improve the efficacy of DEN DNA vaccines.

Immune responses to DNA vaccines have been enhanced by co-administering them with immune-modulating compounds including cytokines, chemokines, co-stimulatory molecules and cationic lipids. Although these approaches have resulted in improved potency, none of these are approved for use in humans with prophylactic vaccines. Aluminum-based adjuvants have been used successfully in humans to enhance the immune response to protein vaccines and are approved for use in humans. Studies have demonstrated that intramuscular co-administration of DNA vaccines with aluminum phosphate enhanced the antibody response 10- to 100-fold. This response occurred with DNA vaccines for hepatitis B and influenza A. The use of calcium phosphate, aluminum hydroxide and aluminum hydrophosphate as adjuvants had an inhibitory effect on the antibody response to the DNA vaccines. This inhibitory effect was the result of the DNA binding to these adjuvants, making less available for uptake by antigen presenting cells (APCs). The enhancement of vaccine potency was the result of increased recruitment of APCs.

Highly immunogenic carriers such as tetanus toxoid, diphtheria toxoid and cholera toxin have been used successfully to enhance the immunogenicity of polysaccharide vaccines. These proteins work by involving T-cells in the processing of the carrier protein (T-cell help) and by generating both T- and B-cell memory. Little is known about the effect of these carrier proteins on the immune response to DNA vaccines.

Another possible way to enhance the uptake, expression and processing of vaccines is to link them with antibodies that more efficiently direct them to APCs. Gosselin et al. cross-linked anti-FcγRI monoclonal antibodies with tetanus toxoid and compared the immunogenicity of this compound with that of tetanus toxoid alone. The results showed that by linking the immunogen with the monoclonal antibody, antigen presentation was increased 100-fold. The mechanism of the increased antibody response was thought to involve the more efficient uptake and processing of the tetanus toxoid by APCs. This approach has yet to be attempted using DNA vaccines as the immunogen. Because only a small portion of DNA vaccines is actually taken up and expressed by APCs, it stands to reason that increasing the efficiency of uptake would result in enhanced cellular and humoral immune responses to nucleic acid vaccines.

This proposal seeks to enhance the immune response to a dengue (DEN) prM/E DNA vaccine by utilizing aluminum phosphate, tetanus toxoid and anti-FcγR monoclonal antibodies as adjuvants in various formulations. Phase I of the project will be conducted in mice to demonstrate the feasibility of enhancing anti-DEN immune responses with these novel approaches. Phase II will be conducted using non-human primates to

evaluate the protective efficacy of DNA vaccine formulations that are shown to be immunogenic in mice.

BODY

The overall objective of the project is to use adjuvants to enhance the immunogenicity of a dengue DNA vaccine. The adjuvants to be evaluated in this project include aluminum phosphate, tetanus toxoid and anti-FcγR monoclonal antibodies chemically linked to the DNA vaccine. The first task was to link the dengue DNA vaccine to anti-FcγR monoclonal antibodies. The protocol for linking the monoclonal antibodies to the dengue DNA vaccine was finalized. It was decided to use the method of Stetsenko and Gait (*Nucleosides, Nucleotides & Nucleic Acids*, 20:801-804) to accomplish this. Prior to performing the chemical linking procedure, evaluation of the immunoreactivity of the monoclonal antibodies for mammalian FcγR was performed. The first set of anti-FcγR antibodies was obtained from Accurate Chemical, Wesbury, NY. Anti- FcγRII and FcγRIII were also obtained and evaluated. Indirect immunofluorescence assays (IFA) were performed to evaluate the recognition of Fcγ receptors on K562 cells (human monocyte cell line) and P388D1 cells (mouse monocyte cell line). Experimental results indicated poor recognition of mouse and human Fcγ receptors by these monoclonal antibodies. A second set of anti-FcγRI and anti-FcγRII monoclonal antibodies was obtained from Pharmingen Inc., San Diego, CA and evaluated against the same cell lines. IFA confirmed recognition of the anti- FcγRII receptors but not FcγRI receptors on K562 cells. The P388D1 mouse cells showed no reactivity with either antibody. Antibody binding is currently being confirmed by flow cytometry analysis. If confirmed, the dengue DNA vaccine will be conjugated to the FcγRII monoclonal antibody from Pharmingen Inc.

The original work plan was to evaluate the anti-FcγR/DNA vaccine compound in mice along with the adjuvants aluminum phosphate and tetanus toxoid. Because of the lack of immunoreactivity with the mouse monocyte cell line seen to date, it may be necessary to omit the anti-FcγRI/DNA study arm and evaluate its potential as a vaccine for non-human primate studies based on *in vitro* analysis of uptake and dengue protein expression. Efforts are currently underway to obtain a third set of anti-Fcγ monoclonal antibodies that will demonstrate immunoreactivity with the mouse monocyte cell line.

After numerous revisions, the animal use protocol for mouse testing has been submitted to the NMRCD IACUC and is pending approval. The mouse experiments will begin upon final approval of the protocol.

KEY RESEARCH ACCOMPLISHMENTS

Anti-FcγR antibodies that adequately recognize Fcγ receptors on the monocyte cell line K562 were identified.

Anti-FcγRII monoclonal antibodies react more strongly than FcγRI antibodies. Anti-FcγRII antibodies will therefore be used in the project.

Anti-FcγR antibodies failed to recognize the mouse monocyte cell line P388D1.

The protocol for linking anti-FcγR monoclonal antibodies to the dengue DNA vaccine was finalized.

The animal use protocol was modified per IACUC member and veterinary personnel recommendations and is pending final approval.

REPORTABLE OUTCOMES

There are no reportable outcomes for this project at this time.

CONCLUSIONS

Anti-FcγRI monoclonal antibodies poorly recognize FcγRI receptors on K562 cells. In contrast, anti-FcγRII monoclonal antibodies demonstrate immunoreactivity with K562 FcγRII receptors. Neither antibody recognized receptors on P388D1 cells. The anti- FcγRII antibodies will be linked to the dengue DNA vaccines and evaluated for uptake and expression in the K562 cell line.

REFERENCES

Stetsenko, DA and Gait, MJ. A new "native ligation" procedure for peptide-oligonucleotide conjugation. Nucleosides, Nucleotides & Nucleic Acids. 2001. 20:801-804.